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Anesthetic Preconditioning

Effects on Latency to Ischemic Injury in Isolated Hearts

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Background: **Anesthetic preconditioning (APC) is protective for several aspects of cardiac function and structure, including left ventricular pressure, coronary flow, and infarction. APC may be protective, however, only if the duration of ischemia is within a certain, as yet undefined range. Brief ischemia causes minimal injury, and APC would be expected to provide little benefit. Conversely, very prolonged ischemia would ultimately cause serious injury with or without APC. Previous investigations used a constant ischemic time as the independent variable to assess ischemia-induced changes in dependent functional and structural variables. The purpose of the study was to define the critical limits of efficacy of APC by varying ischemic time.**

Methods: **Guinea pig hearts (Langendorff preparation; n = 96) underwent pretreatment with sevoflurane (APC) or no treatment (control), before global ischemia and 120 min reperfusion. Ischemia durations were 20, 25, 30, 35, 40, and 45 min.**

Results: **At 120 min reperfusion, developed (systolic–diastolic) left ventricular pressure was increased by APC compared with control for ischemia durations of 25–40 min. Infarction was decreased by APC for ischemia durations of 25–40 min, but not 20 or 45 min. APC improved coronary flow and vasodilator responses for all ischemia durations longer than 25 min, and decreased ventricular fibrillation on reperfusion for ischemia durations longer than 30 min.**

Conclusions: **Although APC protects against vascular dysfunction and dysrhythmias after prolonged ischemia, protection against contractile dysfunction and infarction in this model is restricted to a range of ischemia durations of 25–40 min. These results suggest that APC may be effective in a subset of patients who have cardiac ischemia of intermediate duration.**

ANESTHETIC preconditioning (APC) is the phenomenon whereby brief exposure of the heart to a volatile anesthetic leads to a state of resistance to the effects of ischemia and reperfusion.[1–6] APC has been shown to be protective for several variables, including contractile function, coronary flow, free radical release at reperfusion, and infarct size. This protection is often described as an improvement in one or more of these variables when hearts were subjected to a fixed duration of ischemia, usually 30 min in experimental models that use global ischemia[3–6] or 30–60 min in those that use regional ischemia.[1,2] The protection afforded by APC is considerable under these conditions, *e.g.,* developed pressure is improved approximately 40–70% and infarct size is reduced approximately 40–60%. Such findings have led to the suggestion that APC may find clinical application.[7]

Nonetheless, if ischemia is sufficiently prolonged, infarction will eventually occur with or without APC. If APC is to find clinical utility, it will most likely be because the additional time it affords before occurrence of dysfunction and/or infarction will allow either spontaneous reperfusion or application of therapies such as angioplasty to relieve a coronary occlusion. In addition, as APC is likely to provide little benefit if ischemia is of brief duration, a window period during ischemia may be postulated to exist wherein APC is effective.

To our knowledge, all previous investigations of APC have used ischemic time as the independent variable to assess ischemia-induced changes in functional and structural parameters. A more meaningful measure of the efficacy of APC may be the additional time it affords during ischemia and reperfusion before critical cellular events occur. The purpose of this study was to evaluate the magnitude of the protective effect of APC and to measure the range of ischemia durations against which APC provides protection. Sevoflurane was used to achieve APC in isolated guinea pig hearts that were subjected to global ischemia.

Methods

The investigation conformed to the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health No. 85–23, revised 1996). Approval was obtained from the Medical College of Wisconsin animal studies committee. Our Langendorff preparation has been described in detail previously.[3–6,8] Guinea pig hearts (n = 96) were perfused at constant pressure (55 mmHg) and at 37°C with an oxygenated Krebs-Ringer solution of the following composition (in mM): Na^+ 138, K^+ 4.5, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134, HCO_3^- 14.5, $H_2PO_4^-$ 1.2, glucose 11.5, pyruvate 2, mannitol 16, EDTA 0.05, and insulin 5 U/L.

Left ventricular pressure (LVP) was measured isovolumetrically using a transducer connected to a saline-filled latex balloon placed in the left ventricle through an

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incision in the left atrium. Coronary inflow was measured by an ultrasonic flowmeter (Transonic T106X, Ithaca, NY). Atrial and ventricular bipolar leads were used to measure spontaneous heart rate. Coronary inflow and coronary venous Na^+, K^+, Ca^{2+}, Po_2, pH, and Pco_2 were measured off-line with an intermittently self-calibrating analyzer (Radiometer ABL 505, Copenhagen, Denmark). Coronary sinus Po_2 tension (PvO_2) was also measured continuously on-line with a Clark electrode (model 203B, Instech; Plymouth Meeting, PA). Myocardial O_2 consumption (Mvo_2) was calculated as coronary flow/heart weight (g) · (Pao_2 –PvO_2) · 24 ml O_2/μl at 760 mmHg. Sevoflurane was bubbled into the perfusate using an agent-specific vaporizer. Sevoflurane concentrations in Krebs-Ringer solution were measured by gas chromatography from samples taken anaerobically from the inflow line just proximal to the flow probe.

Global ischemia was achieved by clamping the aortic inflow line. If ventricular fibrillation occurred on reperfusion, a bolus of lidocaine (250 μg) was given immediately. At 60 min reperfusion, endothelium-dependent coronary vasodilation and endothelium-independent coronary vasodilation were tested by infusing hearts for 3 min with 10 μM bradykinin and 100 μM sodium nitroprusside, respectively. The sequence of bradykinin or sodium nitroprusside administration was randomized for each heart with a 30-min washout period between drugs. At the end of 120 min reperfusion, hearts were removed, cut into 6 transverse sections, and stained with 1% 2,3,5-triphenyltetrazolium chloride in 0.1 M KH_2PO_4 buffer (pH 7.4, 38°C) for 10 min. Infarct size was expressed as a percentage of total ventricular weight.[3–5,8]

There were six ischemia durations (20, 25, 30, 35, 40, and 45 min). Hearts were randomly assigned to ischemia duration (n = 16 per ischemia duration) and further randomized to one of two groups: anesthetic preconditioning (APC) (n = 8 per ischemia duration) or control (n = 8 per ischemia duration). Thus, a total of 96 hearts were used (8 per ischemia duration per treatment).

Hearts in the control groups underwent 20–45 min ischemia and 120 min reperfusion. Hearts in the APC groups were also subjected to a preconditioning protocol consisting of two 5-min pulses of sevoflurane with an intervening 5-min perfusion period with Krebs-Ringer solution and followed by a 20-min perfusion period before 20–45 min ischemia and 120 min reperfusion. We chose this preconditioning protocol because we previously found that it provides significant cardioprotection.[4] Inflow sevoflurane concentration for all groups was 0.53 ± 0.02 mM (SEM) (or 3.6%) at 37°C (no difference among groups exposed to sevoflurane).

Statistical Analysis

Data were expressed as means ± SD or median and twenty-fifth and seventy-fifth percentiles, as indicated. Normally distributed data were compared using two-way analysis of variance with repeated measures on one factor (reperfusion interval) and the second factor being treatment group (APC or control). This was performed at the following time points: baseline (0 min), during and after the preconditioning stimuli, during reperfusion at 5-min intervals to 15 min reperfusion, at 30 min reperfusion, prior to administration of bradykinin or sodium nitroprusside (at 60 min and 90 min, randomized), and at 120 min reperfusion. Responses to vasodilators were measured 3 min after initiating administration. *Post hoc* Student-Newman-Keuls tests were used where differences were found (Prism version 3.0a, GraphPad Software Inc, San Diego, CA). Ventricular fibrillation data were not normally distributed and are presented as median and twenty-fifth and seventy-fifth percentiles. Comparisons between control and APC groups were performed using the Fisher exact test. Differences among means were considered statistically significant when $P < 0.05$ with correction for multiplicity. All P values were two-tailed.

Results

Baseline values (systolic and diastolic LVP, coronary flow, heart rate, Mvo_2) were similar for all groups. Figure 1 shows developed (systolic–diastolic) LVP for control and APC groups at baseline and during reperfusion after ischemia of each duration. When ischemia duration was 20 min, recovery of developed LVP was similar in control and APC groups (fig. 1, *A*). When ischemia duration was 25, 30, 35, or 40 min, recovery of developed LVP was better in the APC group (fig. 1, *B–E*). When ischemia duration was 45 min, developed LVP was similarly impaired in control and APC groups (fig. 1, *F*). Differences between APC and control groups in developed LVP was primarily due to decreases in systolic LVP, as diastolic LVP (compliance) was improved at 120 min reperfusion in the APC group after 40 and 45 min ischemia (Table 1). For all ischemia durations in APC and control groups, developed LVP was significantly impaired at 120 min reperfusion compared with baseline.

Figure 2 shows coronary flow at baseline and during reperfusion after ischemia. When ischemia duration was 20 or 25 min, coronary flow recovered equally in control and APC groups, and at 120 min had recovered to baseline values (fig. 2, *A* and *B*). Reperfusion hyperemia was evident in the APC, but not in the control group, after 25 min ischemia (fig. 2, *B*). When ischemia duration was 30, 35, 40, and 45 min, recovery of coronary flow was greater in the APC group than in the control group (fig. 2, *C–F*).

Figure 3 shows increases in coronary flow with sodium nitroprusside (fig. 3, *A*) and bradykinin (fig. 3, *B*), administered at 60 and 90 min reperfusion (in random order), following ischemia of varying duration. Data are ex-

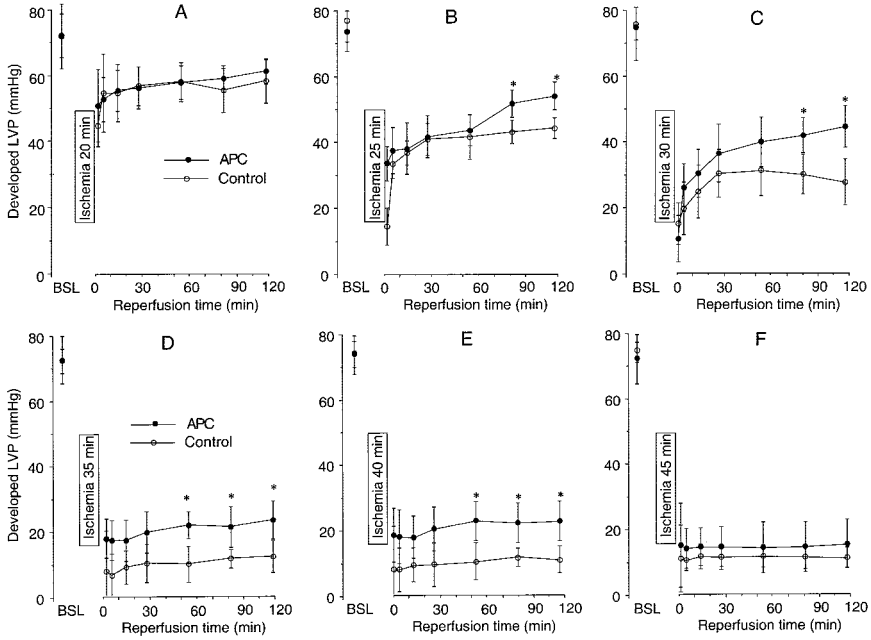

Fig. 1. Developed (systolic–diastolic) left ventricular pressure (LVP) for anesthetic preconditioning (APC) and control groups, at baseline (BSL), and during 120 min reperfusion after ischemia of the following durations: (*A*) 20 min, (*B*) 25 min, (*C*) 30 min, (*D*) 35 min, (*E*) 40 min, (*F*) 45 min. n = 8 hearts per ischemia duration/treatment. Mean ± SD. * $P < 0.05$, APC *versus* control.

pressed as percentage increases from basal coronary flow at 60 and 90 min reperfusion. For both these agents control and APC groups showed similar vasodilator responses after 20, 25, and 30 min ischemia, whereas responses were increased to both these agents in the APC group compared with the control group after 35, 40, and 45 min ischemia.

The only dysrhythmia observed on reperfusion was ventricular fibrillation, which occurred in both groups after each ischemia duration. There were no differences between control and APC groups, at any of the ischemia durations, in the proportion of hearts that sustained one or more episodes of ventricular fibrillation during reperfusion. However, the median number of episodes of ventricular fibrillation per heart was decreased in the APC group compared with control group, following 35, 40, or 45 min ischemia (fig. 4). Increases in the median number of episodes of ventricular fibrillation per heart in each group as ischemia duration was increased were not significant in the APC group, but were significant in the control group ($P < 0.05$, chi-square test for trend). Timing of onset of ventricular fibrillation varied from 0–80 min reperfusion. There were no differences in the timing of onset of ventricular fibrillation episodes at any

Table 1. Diastolic LVP, before (Baseline), during (APC Pulse), and after (Washout) Preconditioning Stimuli, and on Reperfusion after Ischemia of Duration 20–45 min

Ischemia Time Line	Baseline (0)	APC Pulse (0)	Washout (40)	RP 60 min (130)	RP 120 min (190)
20-min					
Control	1 ± 2	1 ± 2	1 ± 3	2 ± 4	1 ± 3
APC	1 ± 1	1 ± 1	0 ± 1	2 ± 4	2 ± 4
25-min					
Control	0 ± 1	0 ± 1	0 ± 3	7 ± 5	5 ± 6
APC	1 ± 1	0 ± 2	1 ± 1	7 ± 5	4 ± 4
30-min					
Control	1 ± 2	1 ± 2	1 ± 3	18 ± 6	14 ± 7
APC	1 ± 1	1 ± 2	2 ± 2	10 ± 4*	9 ± 4
35-min					
Control	0 ± 1	0 ± 1	0 ± 2	22 ± 3	19 ± 3
APC	1 ± 2	3 ± 2	0 ± 4	12 ± 7*	8 ± 5*
40-min					
Control	0 ± 1	0 ± 1	0 ± 1	23 ± 6	20 ± 7
APC	0 ± 0	0 ± 3	0 ± 1	12 ± 6*	11 ± 7*
45-min					
Control	0 ± 1	0 ± 1	0 ± 1	19 ± 5	20 ± 6
APC	0 ± 2	0 ± 2	0 ± 2	11 ± 5*	13 ± 5*

Values are mean ± SD; n = 8 per ischemia duration/treatment.

* $P < 0.05$ APC *vs.* Control.

APC = anesthetic preconditioning group; LVP = left ventricular pressure; RP = reperfusion.

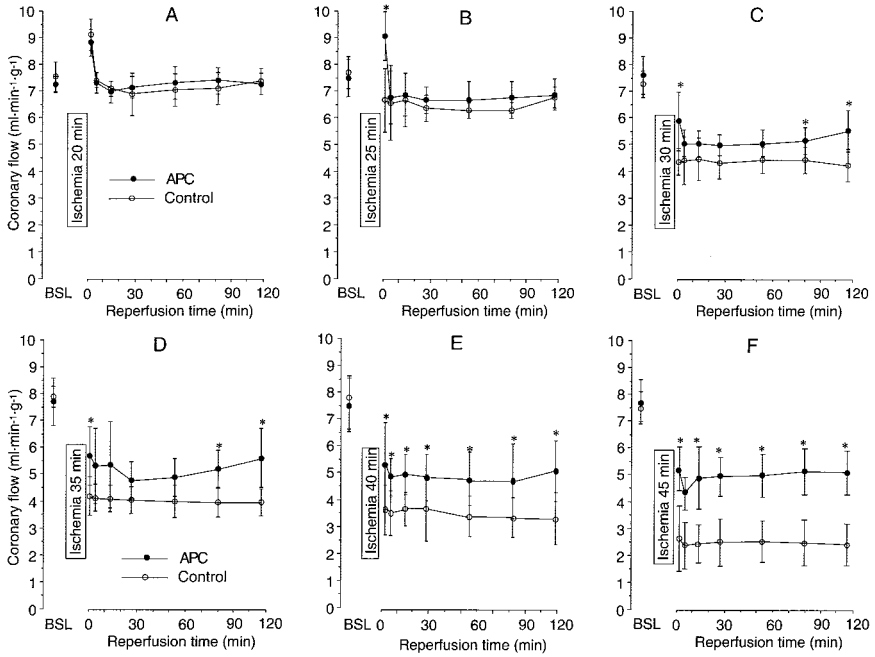

Fig. 2. Coronary flow for anesthetic preconditioning (APC) and control groups, at baseline (BSL), and during 120 min reperfusion after ischemia of the following durations: (*A*) 20 min, (*B*) 25 min, (*C*) 30 min, (*D*) 35 min, (*E*) 40 min, (*F*) 45 min. Mean ± SD. * $P < 0.05$, APC *versus* control.

of the ischemia durations, or between control and APC groups (data not shown).

Figure 5 shows infarct size, expressed as a percentage of total ventricular weight. Differences between control and APC groups were significant when ischemia duration was 25–40 min, but there were no differences when ischemia duration was 20 or 45 min.

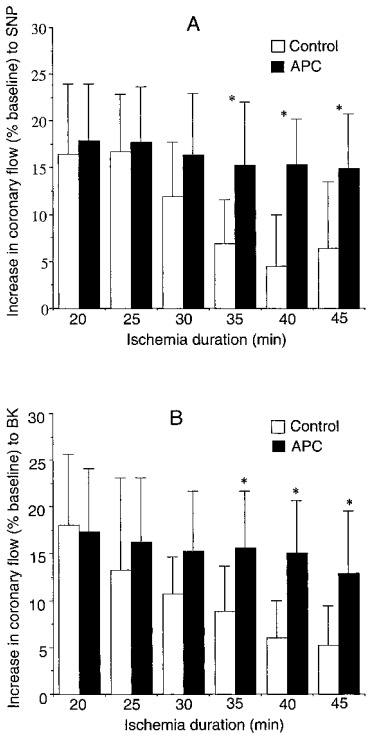

Fig. 3. Vasodilator responses to (*A*) sodium nitroprusside (SNP) and (*B*) bradykinin (BK) for anesthetic preconditioning (APC) and control groups, expressed as a percent increase in coronary flow from prevasodilator levels. SNP and BK were administered at 60 and 90 min reperfusion (in random order), after ischemia of variable duration (20–45 min), as indicated. n = 8 hearts per ischemia duration/treatment. Mean ± SD. * $P < 0.05$, APC *versus* control.

Discussion

Previous exposure to a volatile anesthetic protects the heart from the effects of ischemia/reperfusion injury.[1–6,8] Whereas previous studies have examined protective effects following fixed periods of ischemia, a more meaningful measure of the efficacy and potential clinical utility of APC may be the incremental time during ischemia that APC affords before irreversible ischemic damage occurs. In this study, we examined effects of APC on several parameters of cardiac function, and on infarction, after a range of ischemia durations. We found that protection against contractile dysfunction and infarction

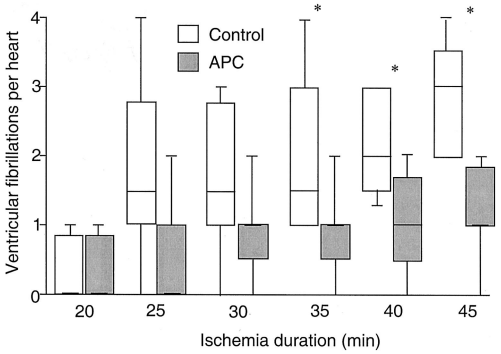

Fig. 4. Box plot of ventricular fibrillation during reperfusion after ischemia of variable duration (20–45 min), as indicated. n = 8 hearts per ischemia duration/treatment. Box plot represents median (*central line*), twenty-fifth–seventy-fifth percentiles (*box*), and range (*whiskers*). * $P < 0.05$, APC *versus* control.

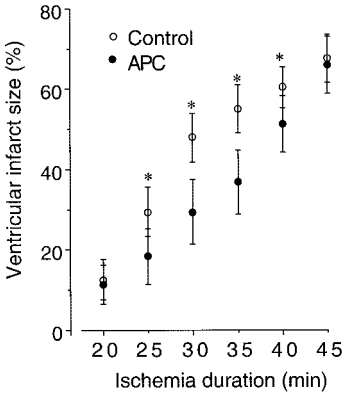

Fig. 5. Ventricular infarct size at 120 min reperfusion expressed as a percentage of ventricular weight. n = 8 hearts per ischemia duration/treatment. Mean ± SD. *$P < 0.05$, APC *versus* control.

followed a bell-shaped distribution, with no significant protective effects when ischemia duration was 20 min or 45 min, and an intervening period wherein APC was effective. Thus, in this model, APC is effective only for a limited range of ischemia durations, and even for ischemia durations for which APC is maximally protective, the additional ischemic time that APC allows before injury is similar to unpreconditioned hearts is quite short—on the order of 10 min for infarct size and developed LVP. In contrast, protection against diastolic contracture and coronary vascular injury did not follow such a distribution, because protection was demonstrable when ischemia durations were longer than 20 min, including the longest duration of ischemia studied, 45 min.

Since the first description of preconditioning,[9] experimental studies have focused primarily on elucidating cellular mechanisms, and have therefore chosen an ischemia duration that was likely to demonstrate differences in preconditioned and unpreconditioned hearts. A literature review revealed that ischemia duration is most often 30 min in models using global ischemia and 30–60 min in those using regional (coronary ligation) ischemia. The longer ischemia duration sometimes chosen in regional ischemia models reflects the effect of collateral flow to limit subsequent infarction and contractile dysfunction.[10] In those studies preconditioning-induced protection was expressed in terms of improved functional and structural variables, primarily developed LVP and infarct size. In the clinical setting however, duration of ischemia cannot be accurately predicted. To our knowledge, no previous studies have specifically examined the time span of ischemia over which APC is protective.

Short periods of ischemia cause little injury, and in such cases APC is unlikely to be of benefit. This was demonstrated by Jenkins *et al.,*[11] who reported that ischemic preconditioning (IPC) produced a 57% reduction in infarction prior to 20 min ischemia, whereas no reduction of infarction was achieved if ischemia duration was 15 min. Conversely, very prolonged periods of ischemia would be expected to cause substantial infarction with or without preconditioning. Murry *et al.*[9] demonstrated that whereas IPC prior to 40 min regional ischemia limited infarct size by 75% compared with the control group, no difference was seen when the ischemic time was 3 h. Thus, at these extremes, IPC may be expected to cause no change in outcome, with an intervening window period wherein preconditioning is effective. It is difficult to compare global and regional models of ischemia for IPC. However, the lower point of this window of protection is likely to be close to 20 min irrespective of the model, because ischemia of less than 20 min causes minimal injury.[12] Previous literature allows only speculation about the likely upper point of the window period. Jenkins *et al.*[11] found that IPC produced more protection against 20 min global ischemia than against 30 min global ischemia (57 and 37% reduction in infarction, respectively); similarly, Uematsu *et al.*[13] found a greater reduction in infarct size by IPC after 20 min compared with 30 min global ischemia (73 and 50%, respectively). Therefore, in those studies the effects of IPC on infarct reduction were diminished substantially when ischemia reached 30 min.

We sought to identify both extremes of the preconditioning window period induced by sevoflurane. Recently, we reported increases in mitochondrial Ca^{2+} and nicotinamide adenine dinucleotide,[5,6] and in reactive oxygen species during ischemia[4] and reperfusion,[3] and amelioration of these values by APC. The profiles of these changes in anesthetic preconditioned hearts suggest that if ischemia were approximately 10 min longer, values of these variables would reach levels found in control hearts. Although we cannot determine exactly from the present results the reason why prior anesthetic exposure failed to protect against injury after longer ischemia, it is likely that mitochondrial electron transport chain dysfunction—manifested by accumulation of nicotinamide adenine dinucleotide and mitochondrial Ca^{2+} and formation of reactive oxygen species—is delayed rather than prevented by APC. Impaired mitochondrial energetics on reperfusion will ultimately be a major determinant of contractile function and tissue viability.

Our results indicate that APC affords a broad spectrum of protective effects, including improved contractile and vascular function, and decreased dysrhythmias and infarction. Protective effects on infarct size and contractile dysfunction followed a bell-shaped distribution for ischemia duration. In contrast, protective effects on vascular dysfunction did not follow a bell-shaped distribution but were consistent for ischemia durations longer than 25 min. Furthermore, vasodilator effects of bradykinin and sodium nitroprusside were greater after 45 min ischemia in the APC group compared with the control group, indicating that endothelium-dependent and endothelium-independent relaxation were better preserved.

Ischemia and reperfusion is known to induce struc-

tural injury in endothelial cells[14] and to impair endothelium-dependent vasodilation of coronary vessels.[15] IPC[16,14] and APC[17] have been shown to protect vascular responsiveness in hearts subjected to ischemia. Isolated endothelial cells are preconditioned by hypoxia.[18] Indeed, preconditioning mechanisms are similar, at least in some respects, for endothelial cells and cardiomyocytes.[19,20] However, it is difficult to determine in intact heart models if improved vascular function partly or wholly reflects preconditioning of endothelial cells in addition to cardiomyocytes, or if improved perfusion reflects decreased myocardial infarction and the accompanying decreased extravascular compression or other secondary effects. We found that diastolic compliance was improved in the APC group during reperfusion after 40 and 45 min ischemia; thus, decreased myocyte contracture may have been responsible for the improved coronary flow. It has been suggested that endothelial injury does not occur during ischemia but is purely a manifestation of reperfusion.[16] Our results do not support this hypothesis, because more prolonged ischemia resulted in progressively severe vascular dysfunction in control hearts.

The differential effects of APC we observed between vascular function *versus* contractile function and infarction could reflect decreased metabolic demands of the coronary vasculature compared with the myocardium. This may allow the vasculature to better tolerate prolonged ischemia, as demonstrated by the rightward shift in the ischemia duration/function curve for flow and better vasodilator responsiveness. It could also reflect a radical-scavenging effect of nitric oxide that reacts with the superoxide radical to limit formation of highly reactive hydroxyl radical. Cardiomyocytes produce minimal quantities of nitric oxide compared with endothelial cells. Importantly, with regard to the isolated heart model for study of APC, the observed "disconnection" between coronary flow and infarction confirms that previously reported protective effects on infarction were not merely secondary to changes in coronary vascular perfusion.[3–6,8]

Dysrhythmias, *i.e.*, ventricular fibrillation, were similarly reduced by APC for ischemia durations longer than 30 min. This is in agreement with the findings of Lawson *et al.*,[21] in a study of the antidysrhythmic effect of IPC in isolated rat hearts. They reported a reduced incidence of ventricular dysrhythmias across a broad range (10–40 min) of ischemia durations. Interestingly, they found that whereas a therapy that delays progression of ischemic damage would be expected to delay the onset of reperfusion dysrhythmias,[22] the timing of dysrhythmia onset was unaltered by IPC. They proposed that the antiarrhythmic effect of IPC is independent of its antiischemic effects. Our dysrhythmia results are consistent with their hypothesis, as the timing of onset of dysrhythmias was similar in APC and control groups. Unfortunately, Lawson *et al.*[21] did not report other functional or structural variables to allow further comparison with our results.

Limitations to this study include the limited number of different ischemia durations tested; this necessarily limits the accuracy of our estimation of APC effect. However, our major aim was to identify extremes at which APC failed to protect and to describe the shift in the curves describing functional and structural protection by APC. The few previous studies that examined the time-dependent, infarct-limiting effect of preconditioning all pertained to IPC[9,11,13] and did not attempt to define the lower and upper limits of the protective effect. Our selection of 5-min incremental ischemia durations, although somewhat arbitrary, was sufficient to derive a clear graded response. More importantly, it is difficult to compare experimental models of global and regional ischemia and to extrapolate results to human myocardial ischemia, which is generally regional with highly variable degrees of collateralization. We surmise that in humans the ischemia duration/injury curve after APC would be shifted to the right compared with the model described here, particularly in patients with high levels of collateralization.

In summary, APC differentially protected several facets of myocardial performance, but contractility and infarction will ultimately be the important limiting factors for potential clinical efficacy of APC. Overall, our results suggest that APC is effective, provided ischemia is of the appropriate duration. If the typical duration of ischemia during coronary artery bypass falls within this range, APC may prove to provide highly useful therapy, but in patients who experience more prolonged ischemia, APC is unlikely to be of benefit.

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